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The Wound-Healing Process

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INTRODUCTION

Diabetes is on the rise in the United States and the rest of the world, and its complications are even more evident in the aging population. Among the most severe complications of diabetes are impaired circulation and wound healing. The former condition, together with peripheral neuropathy, contributes to an insensate, poorly vascularized lower extremity that is prone to the development of chronic wounds. Lack of sensation leads to aggravation of the injury, which can frequently lead to the spread of infection and the loss of all or part of the lower limb. The circulatory defects occur in both conducting vessels—which are prone to atherosclerosis—and the microcirculation, which shows signs of basement membrane thickening and diminished reparative capacity. Surgical intervention can sometimes alleviate the macrovascular defects, but grafting procedures cannot guarantee that tissue perfusion can be restored. With the exception of the retina, the poor growth of new capillary vessels in diabetes broadly diminishes the capacity to repair.

WORKING DEFINITIONS

For the purposes of this discussion, we define wound repair as the effort of adult tissues to restore normal tissue function and architecture after one of a variety of physical, mechanical, biological, or chemical insults. The primary demands of a system that is undergoing wound repair are the restoration of normal blood and lymphatic circulatory patterns, the formation of a surface barrier to fluid loss and further infection, the suppression and elimination of foreign organisms and material from the injury site, and reestablishment of mechanical integrity. Often these events occur with great urgency; thus, perfect reorganization is sacrificed in the name of adequate function. On the other hand, regeneration implies the complete restoration of pre-existing tissue architecture and all cellular elements in the absence of scar formation. Although regeneration is an ideal in the wound-healing world, it is typically only found in embryonic development, lower organisms, or in restricted tissue compartments such as bone. Angiogenesis is a crucial element of wound repair. In its broadest sense, it is defined as the formation of new blood vessels from a pre-existing, surrounding blood supply. In the healing wound, new blood vessel formation is a rapid and

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transient phenomenon, which occurs during the proliferation of granulation tissue. Fibrosis refers to the excess formation of extracellular connective tissue that often accompanies wound repair processes. Excess of connective tissue accumulation may go beyond the function of restoring mechanical integrity and act to impede the normal architecture and physiological function of the injured tissue. Fibrosis in the skin can result in mild disfigurement or severe loss of mobility.

HISTORY

The record of wound-healing analysis and therapy goes back many centuries. Works by Majno *(1)* and Needham *(2)* give very nice accounts of the earliest efforts in wound repair up to and including the modern era. Wound healing has always been a major interest of the surgeon, and, with the discovery of antiseptic procedures and antibiotics, the extent to which surgical solutions to wound-healing problems can be applied has grown enormously. Nevertheless, there are a wide range of wound-healing complications which do not respond to simple, surgical treatment: nonhealing wounds, wounds that heal excessively, and wounds that become locked in uncontrolled growth (tumors). With the advent of modern biochemical and molecular techniques, a new perspective of the process has been opened before us *(3,4)*. These discoveries have, in turn, created a new prospect for our understanding and treatment of the problem wound *(5)*.

TERMINOLOGY

There are a few general terms that should be described before dealing with specific issues of wound repair. The term cytokines is used to describe a collection of small protein molecules that are used to provide communication between cells of the inflammatory system. Perhaps the best known of this group are the interleukins, leukocyte intercellular signaling molecules; however, there are also a wide variety of other secreted gene products which act on specific receptors to induce responses such as cell movement, cell growth, or even cell death. In contrast to cytokines, which are frequently involved in regulating inflammation, a distinct group of proteins crucial to the repair process are the cellular growth factors. As implied by their name, the principal function of these proteins molecules is thought to be the promotion of cell and tissue growth; in addition, these molecules can also act to stimulate cell movement and cell migration. Some of the growth factors also stimulate cells to produce more connective tissues. All these molecules can be thought of as hormones that act at the local tissue level to bring about specific tissue responses. As such, they act in autocrine (on like cells), paracrine (on unlike cells), or juxtacrine (on nearby cells) fashion depending on the nature of the target cell.

A crucial class of proteins involved in tissue repair is the proteinases. These are protein-degrading enzymes that can be released by both inflammatory and connective tissue cells. When proteinases act outside the cell, they are crucial for degradation of foreign material, for promoting cell movement through tissue spaces, and for tight regulation of the abundance and distribution of various molecules in the extracellular space. Some proteinases are also important catalysts for further enzymatic reactions.

COMPONENTS AND MEDIATORS IN THE SKIN

Extracellular Matrix

The integrity and mechanical properties of every tissue are governed by its extracellular matrix. This consists of a complex mixture of fibrous and adhesive macromolecules that determine such physical characters as tensile strength, elasticity, hydration, and compressibility *(6)*. The best known and most abundant component in this class is collagen, which is a family of fiber-forming proteins characterized by a rope-like arrangement of three individual polypeptide chains, which confers to every collagen molecule a high degree of stiffness, resistance to proteolytic degradation, and highly defined, rigid structure. Collagen molecules characteristically assemble into higher-order fibrils and fibers whose organization is stabilized by covalent chemical cross-links between individual molecules to produce the predominant material of tendons, ligaments, blood vessels, and skin. Some members of the collagen family of proteins serve more specialized functions, such as determining overall fibril diameter or promoting interactions with other components in the extracellular matrix. In addition, several of the collagen types (presently types I–XXVII) have very restricted distributions in either the basement membrane underlying epithelial surfaces or in structures that attach epithelium to underlying connective tissue.

Under physiological conditions, fibrous collagen is only degraded by highly specialized proteolytic enzymes known as collagenases. The collagenases are in turn members of a much larger class of proteinases, the matrix metalloproteinases (MMPs).

Elastin is, as the name implies, a rubber-like protein, which is often found in association with collagen in skin, blood vessels, and other elastic tissues. Unlike collagen, elastin does not form highly organized fibers, perhaps because this would interfere with its function as a rubbery, cross-linked network *(7)*. The principal role of elastin appears to be the development of elastic properties in tissues. Elastin is noteworthy in wound healing because, at least in the skin, it is one of the last components to be replaced during the repair sequence.

Proteoglycans are composite molecules as the name implies. They consist of a core protein to which are attached acidic sugar chains distinctively built up from the addition of disaccharide subunits. These highly charged molecules play an important role in maintaining hydration of tissue spaces, and they also interact strongly with many other components in the extracellular space, including connective tissue proteins and growth factors. Proteoglycans that contain heparan sulfate side chains are particularly significant because they can bind many other molecules.

Because the diabetic state invariably involves hyperglycemia, nonenzymatic glycation of collagen, and other proteins can be extensive. The relatively long half-life of matrix proteins in combination with this modification can lead to the formation of undesirable cross-links among molecules, which reduces solubility of the matrix. In addition to stiffening of blood vessels, hyperglycemia may contribute to atherosclerosis and other vascular complications *(8–12)*. Further investigation is needed to determine if strategies that reduce the formation of these byproducts can reduce the wound-healing deficit in diabetes.

ADHESIVE PROTEINS

Cells, whether they are moving about in tissue or fixed in place, require adhesive interactions with their surroundings. This is accomplished by the interaction of specific cell surface receptors with molecules in the extracellular environment. The classic

example of this system and cell–matrix interactions is the family of integrin molecules. These matrix receptors consist of two subunits that are mainly external to the cell but which transverse the cell membrane and may be closely associated with many elements of the cytoskeleton on the cell interior. On the cell surface, these integrins interact with specific sites in extracellular matrix components to provide adhesion and even some recognition functions. Although integrins can bind to a number of different matrix macromolecules, they are importantly associated with a group of molecules known as cellular attachment factors, most of which are glycoproteins that have branched sugar chains attached to the protein backbone *(3)*. The best known of these adhesive glycoproteins is the molecule fibronectin, a component of plasma, which is also produced in a variant form by cells during wound repair. This molecule has many different interactive regions. Fibronectin can form a bridging link from cells (via integrins) to collagen, to heparin or heparan-containing proteoglycans, and to fibrin. Thus, this glycoprotein is a prototype for the intercellular cement that links many of the elements of the matrix together with its constituent cells. Another prominent plasma attachment factor is the protein vitronectin. Cells themselves produce many attachment and migration factors including osteopontin, osteonectin/SPARC, tenascin, and thrombospondin. In some cases these latter three molecules, depending on the cell type and circumstances, may have anti-adhesive properties.

BASEMENT MEMBRANES

There is highly specialized extracellular matrix organization found in the basement membrane underlying all epithelial cells *(13)*. These structures surround the endothelial lining of all blood vessels and underlie epidermal surfaces. Although they are often very thin and evanescent, basement membranes serve an important function in maintaining the polar organization of the epithelium assuring its appropriate attachment to underlying connective tissue and acting as a physical barrier to large molecular complexes and cells. Basement membranes characteristically contain some unique members of each of the extracellular matrix classes we have already discussed: type-IV collagen, which tends to form lattices rather than extended fibers; laminin—a large, multifunctional, cross-shaped attachment factor, and perlecan—a proteoglycan that is particularly rich in heparan sulfate side chains. Basement membrane collagens do not have a continuous triple helical structure, so they are susceptible to degradation by a wider range of proteinases.

CELL POPULATIONS

A variety of cell populations play a role in wound healing. In the epidermis, the upper layer of the skin, the predominant cells are the keratinocytes. This is a self-renewing cell population in which the basal cells remain closely associated with the basement membrane whereas their daughter cells differentiate and move upward through the epidermis to produce the cornified layers that protect the skin. Other epidermal cells include the Langerhans cell and the dendritic cell, which are involved in host defense and antigen presentation. Lymphocytes can also appear in the epidermis under pathological conditions. Melanocytes produce pigment that is transferred to the keratinocyte. In the deeper layer of the skin, the dermis, the predominant cell type is the fibroblast, which is embedded in

a dense meshwork of extracellular matrix molecules. Blood is brought to the skin by vascular channels lined with endothelial cells. The pericyte is a smooth muscle cell-like component, which is closely associated with capillary endothelial cells. The larger vessels, both arteries and veins, will have surrounding vascular smooth cells. A number of cells of the inflammatory system also reside in the dermis. The mast cell is a key participant in any immunological and allergic reactions, and resident tissue macrophages are also critical to immune surveillance. During injury, many new cell types move from the circulation into connective tissue space. These include leukocytes: macrophages, lymphocytes and neutrophils, and platelets.

CYTOKINES, GROWTH FACTORS, AND THEIR RECEPTORS

Growth factors have powerful, positive actions during the wound-healing process. The prototypical growth factor is epidermal growth factor (EGF). It was originally identified as a small protein produced in the salivary gland of male mice by Stanley Cohen, and it was noted for stimulating the premature opening of eyelids of the fetal mouse. EGF is actually a part of a family of related proteins, which include EGF, transforming growth factor (TGF)-α, betacellulin, heparin-binding EGF, epiregulin, neuregulin, and amphiregulin *(14)*. As true of all the growth factors, these proteins bind to very specific and selective cellular receptors that transmit a set of signals within the cell to stimulate cell growth and division. An unusual feature of the EGF family is that many members actually are synthesized as much larger cell surface proteins with "tails" penetrating into the cell interior. The active forms are then cleaved from the exterior of the cell surface by specific enzymatic activities. EGF has been shown in a number of studies to stimulate wound repair both in fibroblasts and epithelial cells. It has not achieved much clinical success, although it is presently used in some formulations for corneal lubrication.

A second important class of growth stimulating molecules is the platelet-derived growth factors (PDGFs) *(15)*. These molecules are contained in platelet granules, and the release of PDGF at sites of injury during clot formation is certainly an important aspect of the repair process. Commercial preparations of platelet lysates have shown success in clinical application, most likely because of the presence of PDGF and other growth factors. This molecule has been widely implicated in the pathological growth of vascular smooth muscle cells during atherosclerosis, and one of the two peptide chains of PDGF is closely related to a viral (v-*sis*) oncogene. Thus, growth factor-like molecules are involved in growth control of transformed cells. As with EGF, the response to this stimulus is dictated by the presence of specific PDGF receptors. PDGF is produced in two isoforms (A and B) and PDGF molecules exist as AA, AB, or BB forms which are selectively recognized by two different kinds of PDGF receptors.

Two growth factor types are significant for their ability to stimulate angiogenesis. Fibroblast growth factor (FGF) refers to a family of proteins that interact with one or more of four related receptors on a variety of cell types *(16)*. FGF-1 and -2 (acidic and basic FGF) are particularly noteworthy for their ability to stimulate endothelial cell growth and capillary cell invasion in a variety of models. Most forms of FGF have growth effects on many cell populations, but endothelial cells appear to have a greater sensitivity; however, there are two novel forms of FGF (FGF-7 and -10 or keratinocyte growth factors-1 and -2) that specifically stimulate the proliferation and differentiation

of epithelial cells because of unique receptors present on these cells *(17)*. These two growth factors have a classical paracrine mode of action because they are produced in the dermis and act on the epidermis. Vascular endothelial growth factor (VEGF) or vascular permeability factor is the most selective of the growth factors for endothelial action. As first name implies, VEGF acts to stimulate endothelial cell growth and recruitment *(18,19)*. The latter name, vascular permeability factor, refers to an important property of VEGF, the ability to increase capillary permeability and leakage of plasma into tissue space. VEGF has received very recent notoriety for its ability to stimulate the revascularization of ischemic limbs and cardiac muscle *(20)*. It is very likely that this molecule is going to assume an important role in the therapy of wound repair particularly in which blood supply is compromised. VEGF-C is exclusively responsible for the process of lymphangiogenesis, the growth of new lymphatic structures.

Connective tissue growth factor (CTGF) is another, newer growth factor *(21)*. Although this molecule appeared to have some antigenic similarity to PDGF, it is chemically a very different structure. It appears to be highly specific for connective tissue cells such as fibroblasts and chondrocytes. Its expression is associated with sites of tissue repair. CTGF has been reported to stimulate connective tissue formation as well *(21)*. Perhaps more significant is the observation that CTGF is strongly induced by treatment of cells and tissues with another growth factor, transforming growth factor (TGF)-β. Recent findings suggest that CTGF may be the downstream signal molecule that actually carries out the matrix-forming activities of TGF-β.

TGF-β is a part of a very large superfamily of proteins that are involved in development, growth, and differentiation *(22)*. Besides TGF-β itself (there are three different forms in man), other well-known members of this family are the bone morphogenic proteins, activin, inhibin, and Mullerian inhibitory substance. TGF-β plays two very prominent roles in inflammation and repair. TGF-β is a very potent inhibitor of immune reactivity, and it suppresses the activation of the immune system *(23)*. Indeed, in knockout mice that lack the TGF-β1 gene, offspring die early in postnatal life from a massive inflammatory reaction. The second major role of TGF-β is in matrix formation and wound healing, in which TGF-β strongly induces—directly or indirectly—the expression of many connective tissue molecules including collagen, elastin, fibronectin, and some proteoglycans, whereas it suppresses the expression of connective tissue degrading enzymes such as collagenase and related MMPs. TGF-β also increases the expression of proteinase inhibitors that act on both the metalloenzyme class of proteinases (tissue inhibitor of metalloproteinases) and the serine proteinase class, such as plasminogen activator inhibitor-1. Thus, it is no surprise that TGF-β expression is often associated with fibrosis and excessive scarring *(24)*. Administration of TGF-β has been shown to accelerate repair in many wound models, and clinical trials of TGF-β2 and TGF-β3 have been conducted as a treatment for chronic wounds.

TGF-β has a complex biology, because it is secreted from cells as a latent factor, and it is sequestered by a class of molecules called latent TGF-β binding proteins *(25)*. Unlike most of the other growth factors, TGF-β and its family members signal, in part, through the assembly of two distinct receptors that signal to the nucleus, in part, through a rather direct pathway that involves molecules known as SMADs *(26)*.

The insulin-like growth factor (IGF)-I and IGF-II are small proteins structurally related to insulin. They each have their own cognate receptor. Insulin itself can interact with the IGF-1 receptor but at about 100-fold higher concentrations. IGF-1 is a growth factor often associated with bone development as an important downstream target of the action of growth hormone. There are significant circulating levels by IGF-1, but it also can be produced locally. IGF-2 binds to a receptor also known as the mannose-6 phosphate receptor. IGF-II expression is more closely associated with development than with tissue repair. IGF-I bioavailability and action is thought to be tightly regulated by a group of molecules known as the IGF binding proteins (IGF-BPs).

Various members of the IGF-BPs can either facilitate the activity of IGF on its receptor or act as sequestering agents to prevent action. Much of the regulation of IGF could thus be at the level of IGF-BP control *(27)*. IGF-1 may be more limited in availability in diabetic wounds *(28)*. Hepatocyte growth factor/scatter factor is another example of a paracrine growth factor that is produced, in large part, by fibroblasts and interacts with the c-Met receptor on epithelial and vascular endothelial cells. This factor has a number of interesting properties, including the stimulation of cell migration and the production of other, angiogenic factors such as VEGF. It has been shown to enhance wound healing in a diabetic mouse model *(29,30)*.

All of these growth factors are proteins, and therefore their production is dictated by the activity of distinct genes. However, regulation occurs at many levels of growth factor action. First, many of these growth factors are synthesized as larger precursor molecules that are biologically inactive until cleared or released. Second, a number of them are expressed in various isoforms whose structure is regulated by alternative splicing during transcription. Third, alternative splicing generates receptor diversity, particularly in the case of FGF. Fourth, unlike endocrine hormones, these proteins are not generally to be found freely circulating in tissue fluid or plasma. Instead they are frequently associated with other molecules, including those of the extracellular matrix, by either electrostatic interactions or more specific protein–protein interactions. Specific examples include the very tight binding of FGF family members to positively charged proteoglycans such as the heparan sulfate proteoglycans *(31,32)*. TGF-β binds to a cell surface proteoglycan, betaglycan, and has also been reported to interact with a small matrix proteoglycan, decorin, and a component of the elastic fibers, fibrillin. Fibrillin is in turn a member of a larger family of molecules known as the latent TGF-β binding proteins *(25)*. The implication of these observations is that the mere evidence that a growth factor gene is expressed at a tissue site or its addition it to a tissue target does not ensure that the biologically active molecule will reach its appropriate receptor and evoke a tissue response.

CELLULAR COMPONENTS OF THE INFLAMMATORY SYSTEM

Platelets

These cells play a critical role in hemostasis and wound healing. Although platelets are fragments of mature megakaryocytes, they act as independent cells at sites of tissue injury. Aggregation of platelets is predominantly induced by exposure of circulating platelets to collagen. Platelets bind to the collagen through a specific integrin

receptor and begin several steps in their irreversible progression toward formation of a thrombus. Many small molecules are released, in particular byproducts of arachidonic acid metabolism. These molecules are released after activation of enzymes at the platelet surface that modify the structure of phospholipids in the plasma membrane. These biologically active molecules, which include the prostaglandins and leukotrienes, have important effects on underlying vascular endothelial and smooth muscle cells.The platelets, once adherent to the fibrin clot and underlying collagen, will, over the course of several minutes, begin to undergo the process of clot retraction. Simultaneously with these events, the platelet releases two important classes of biologically active molecules: first, it releases additional proteins that facilitate cell adhesion and cell migration, including thrombospondin and fibromodulin; second, the platelet granule is a potent source of several growth factors, especially PDGF, TGF-β, EGF, and TGF-α. Thus, extracts of platelets and blood serum are extremely rich in agents which promote wound repair. Deficiencies of platelets lead to impaired blood coagulation and reduced wound healing.

PDGF (becaplermin) is the only pharmaceutical available for treatment of diabetic foot ulcers, whereas several formulations of autologous platelet extracts have been used as part of diabetic wound-healing protocols *(33)*.

Neutrophils

Once blood loss is controlled by fibrin clot formation and platelet activation, the inflammatory system comes into play. By activation of specific adhesive molecules on the vascular endothelial surface, neutrophils bind to endothelium and rapidly move from the luminal surface of vessels into tissue space. Activation can occur through a number of signals, including cell–cell adhesion or the presence of small, soluble signal molecules. Neutrophils play a critical role in debridement and control of infection. Neutrophil activation leads to the release of reactive oxygen species through the actions of enzymatic pathways leading to production of superoxide free radicals and peroxide. These oxidants have extremely potent antimicrobial activity. To some extent, host cells are protected from the action of these reactive oxygen species by enzymatic systems such as superoxide dismutase and catalase. The second important activity of the neutrophil is the release of several proteinases of the serine proteinase class (trypsin-like enzymes). These include elastase, cathepsin, and proteinases. Each of these enzymes has broad substrate specificity and can bring about massive degradation of proteins in the local environment. The activity of these enzymes is normally counterbalanced by the presence of circulating serine proteinase inhibitors such as α1-antiproteinase, α2-antichymotrypsin, and α2-macroglobulin as well as local expression of secretory leukocyte protease inhibitor. Deficiencies in proteinase inhibitors can lead to an imbalance and excess tissue destruction. The neutrophil also expresses a specific form of collagenase, MMP-8, and it also expresses growth factors such as TGF-β.

Normally, the neutrophil has a limited life-span in the wound-healing process. Many spent neutrophils are found in the overlying wound exudate or the eschar. Neutrophil abundance declines with the removal of foreign material and infectious organisms.

Monocytes

Shortly after neutrophil invasion, the next wave of inflammatory cells to normally enter the wound site is the mononuclear phagocyte. These cells, which circulate as monocytes, rapidly differentiate into macrophages on entry into the tissue space, and they play a crucial role in the coordination of cell activities by their expression of growth factors and cytokines. These cells also continue the process of debridement by engulfment of foreign particles; however, most of the enzymatic activity of macrophages is secreted intracellularly into lysosomal vacuoles. Variations in the extracellular environment for macrophage activation can lead to expression of different classes of cytokines. For example, infection may simulate the expression of tumor necrosis factor-α and interleukin molecules, which tend to perpetuate the inflammatory process. On the other hand, appropriate signals will stimulate macrophages to express a wide variety of growth factors including FGF, TGF-β, and VEGF. The macrophage does express a number of important proteinases involved in wound remodeling, including collagenase and metalloelastase (MMP-12).

Lymphocytes

The classical components of the immune system, lymphocytes are not prominently active in acute wounds, although T-cell involvement in chronic lesions is often seen. These immune effectors can have important modulatory activity on the wound healing process *(34)*, but they appear to play a small role in early restoration of tissue architecture.

Mast Cells

These resident tissue cells that act as a component of the inflammatory system are often activated by allergic or immunological stimuli, but they can also play a role in tissue repair *(35)*. Mast cells and their products have been implicated in a number of chronic fibrotic states. These cells are capable of releasing a variety of cytokines and they are known to express a unique set of proteinases of the serine proteinase class.

SEQUENTIAL EVENTS IN THE WOUND REPAIR CASCADE

Under normal circumstances, wound repair is a highly orchestrated progression of events that involves the interaction of the elements described in the preceding sections. Because of the different cell populations and signals involved in each of the stages, it is often convenient to divide this orchestral work into different movements. As one might imagine the first elements in the healing process involve hemostasis, particularly in the case of traumatic injury. The damaged tissue quickly releases tissue factor and other stimuli such as exposed collagen that activate the coagulation pathway leading to production of a fibrin clot and the accumulation of circulating platelets that generate a complete thrombus. These hemostatic events, together with vasoconstriction, driven by arachidonic acid metabolites, eventually stop blood flow. The trapping of both serum proteins and the discharged contents of platelet granules provides the clotting reaction a rich bed on which cellular invasion may begin to take place.

This initial wound material has been termed the provisional matrix by Clark *(36)*. In molecular terms, this matrix consists of fibrin and serum proteins. The growth factors are released from platelets together with adhesion factors such as thrombospondin, fibromodulin, and the important adhesive protein fibronectin. As cells begin to migrate

out of the normal, surrounding tissue to attempt to restore the damaged architecture, this is the first substrate that cells will see. Obviously, appropriate integrin receptors must be generated on the cell surface of migratory cells to recognize these novel substrates as compared to the normal, intact matrix surrounding the cells.

INFLAMMATION

The formation of the provisional matrix is accompanied by an acute inflammatory response. The first waves of inflammatory cells to reach the injury site are the neutrophils, with their potent antibacterial and tissue debridement repertoire. Neutrophil extravasation begins almost immediately as vascular endothelial cell surfaces surrounding the injury site begin to express adhesive molecules such as VCAM and E-selectin, which in turn interact with circulating leukocyte cell surface molecules. After a gradual slowing down of rolling movement within the smaller channels, these cells will eventually come to adhere to sites where they then diapedese through spaces between endothelial cells or move out into the tissue space through the openings of ruptured capillaries. Under normal circumstances, the flux of neutrophils is rapidly terminated, and as necrotic material is degraded these spent cells move upward out of the wound together with their destroyed contents to become part of the eschar. Excessive infection or foreign material may lead to the accumulation of a cellular exudate or—in the worst case—an abscess. The next cell in succession of inflammatory cells is the macrophage, which acts as the key coordinating factor in many of these wounds. Experiments in which animals have been depleted of macrophages show markedly reduced ability to mount a tissue repair response.

Appropriate inflammation and the appropriate function of inflammatory cells have generally been considered indispensable for successful wound healing. Landmark studies in the early 1970s and 1980s demonstrated that immune cells, particularly macrophages, are critical to wound healing, and the ability of macrophages to modulate angiogenesis and fibroplasia has been firmly established *(37–39)*. However, recent gene knockout studies in the mouse call these findings into question *(67)*. There is little argument that proper leukocyte activity assists in microbial decontamination of wounds. In addition, there are several logical arguments in support of a role for leukocytes in healing, even for sterile wounds. First, phagocytic leukocytes, as well as lymphocytes, produce multiple growth factors that promote the repair process. Second, the cellular death and tissue remodeling that occurs during injury and repair probably requires phagocytic clearance for complete resolution. Finally, studies in a variety of model systems suggest that several specific inflammatory cytokines and molecules, including chemokines and nitric oxide, are critical to wound healing *(40–46)*.

The Sequence of Inflammation Following Tissue Injury

Immediately following injury, innate immune cells at the site of injury initiate an inflammatory response. Cells of the innate immune system, including mast cells, resident macrophages, and some specialized T-lymphocyte populations, stand at the ready, acting as sentinels to respond quickly to tissue damage or microbes. In the skin, keratinocytes are often considered a part of the immune sentinel system as well, as this cell type can quickly respond to stimuli and produces several proinflammatory mediators *(47)*. The response of the innate immune cells to injury is rapid, and an abundance of proinflammatory mediators are produced within the first hour following insult. The mediators

produced by resident innate immune cells in response to injury or insult trigger vascular responses, including vasodilation, endothelial cell activation, and increased vascular permeability. Further, early mediators also assist in recruiting the first wave of circulating leukocytes from the bloodstream into the injured tissue. The pattern of leukocytic infiltration into wounds is similar in progression to other acute inflammatory conditions. Neutrophils are the first leukocyte to be recruited in response to chemotactic mediators derived from platelets and resident innate immune cells, and perhaps by activation of complement *(34)*. If the wound is contaminated, microbial products may also serve as leukocyte chemoattractants. Within a day or two of injury, circulating monocytes also enter the wound and differentiate into mature tissue macrophages *(48)*. Macrophages become the most abundant leukocyte in the wound at this stage. Macrophages are thought to play an integral role in the successful outcome of wound healing through the generation of growth factors that promote cell proliferation and protein synthesis *(49)*. Macrophages also respond to neutrophils and their products, and can recognize and ingest apoptotic neutrophils *(50,51)*. The mast cell also increases in density in the wound bed, with most of the infiltration originating from the adjacent tissue *(52)*. As the leukocyte density within the wound increases, the leukocytes that have been recruited into the wound produce large amounts of cytokines and chemoattractants, amplifying the inflammatory response. In the late inflammatory phase of wound repair, T-lymphocytes appear in the wound bed, and may support the resolution and remodeling of the wound *(53,54)*.

Excessive Inflammation in Diabetic Wounds

Poorly healing wounds, including those of individuals with diabetes, often are characterized by a prolonged and dysregulated inflammatory phase *(55–58)*. In mice, the impaired wound healing that is seen in diabetic animals includes a sustained induction of chemokines and a prolonged persistence of neutrophils and macrophages at the site of the injury *(55)*. The excessive neutrophil content of poorly healing wounds, along with the ability of neutrophils to destroy tissue, has suggested that neutrophils might negatively influence repair. Neutrophils do produce a variety of growth factors that could promote revascularization and repair of injured tissue *(59)*. However, they also produce many enzymes that can induce substantial tissue damage. Neutrophil proteases, such as elastase and cathepsin G, can degrade most components of the extracellular matrix as well as proteins as diverse as clotting factors, complement, immunoglobulins, and cytokines *(60,61)*. Because the extracellular matrix serves as a supporting scaffold for infiltrating cells, modification of the extracellular matrix by neutrophils could have important consequences for repair. A reduction in neutrophil content, therefore, seems likely to improve healing outcomes if bacterial burden is not excessive. This concept has been demonstrated in animal models, as wound closure is accelerated in neutropenic diabetic mice *(62)*.

A number of additional recent studies in several systems bolster the concept that leukocytes can be detrimental to the process *(63–67)*. One remarkable example is that of wound repair in the early fetus. In contrast to adult skin, in which injury repair results in a fibrous scar, the skin of an early to midgestation fetus exhibits rapid and scarless regeneration *(68)*. Remarkably, early fetal wounds exhibit very little, if any, inflammatory response, and also demonstrate specific changes in the levels of members of the TGF

family that appear to correlate with the lack of scar formation *(69–72)*. Scarless repair and altered growth factor production in fetal wounds does not appear to be a function of the fetal environment, as adult skin transplanted into a fetal environment maintains a scar-forming phenotype in response to injury *(73)*. Factors intrinsic to fetal skin, including the inflammatory response, appear to be the most important factors in creating the ideal repair phenotype that is seen in this tissue. Interestingly, more recent studies suggest that reduced inflammation can lead to improved outcomes in adult animals as well. Wounds that are produced in the PU.1 null mouse, a mouse that lacks both macrophages and functioning neutrophils, exhibit little inflammation, and heal quickly with reduced scarring *(67)*.

Together, then, these contemporary investigations of wound inflammation suggest that the therapeutic modulation of leukocyte function might improve wound-healing outcomes. Given the immune dysregulation observed in wounds of persons with diabetes, this therapeutic approach seems likely to have special utility in patients with diabetes. The challenge of such an approach will be to support optimal repair whereas maintaining adequate protection from infection.

GRANULATION TISSUE AND EPITHELIZATION

Within 3–4 days, inflammation has begun to subside and the provisional matrix begins to be replaced by the characteristic organ of tissue repair, granulation tissue. This is a highly cellular, highly vascularized mixture of fibroblasts, endothelial cells, and macrophages that advances into the provisional matrix and begins to lay down a more permanent extracellular matrix that provides much greater mechanical integrity to the wound site. The key processes that occur during granulation tissue formation are (1) fibroplasia, the accumulation of collagen and other matrix molecules; and (2) angiogenesis, the formation of a rich capillary bed to supply the rapidly growing extracellular matrix with adequate nutrient supply. Under normal circumstances, granulation tissue is a transient facet of wound repair, often progressing within less than a week to mature scar tissue. Granulation tissue formation and regression is a classic example of rapid cell growth and cell death. Granulation tissue has some unusual characteristics as compared to normal surrounding connective tissue. It is not innervated and therefore insensate. Its rich vascular supply and the presence of a large number of inflammatory cells provide high resistance to infection. The name granulation tissue reflect cobbled surface texture of this material in healing excisional wounds as capillary buds begin to form from underlying intact connective tissue.

Concurrent with the formation of granulation tissue in superficial wounds is the reformation of an intact epithelial (epidermal) sheet. The epidermis has remarkable self-renewing properties inherent in its growth pattern. When a defect occurs in the epidermis, there is rapid transformation of basal keratinocytes at the wound margin to switch to a migratory phenotype. These basal keratinocytes express connective tissue degrading enzymes which seem to facilitate the movement of migrating cells out over the newly deposited/exposed extracellular matrix to rapidly cover the granulation, tissue, thus insuring a barrier to infection, and to further fluid loss. The activated epidermis is also a rich source of growth factors. Intact epidermis also contains many stored cytokines and growth factors such as IL-1 and TGF-β. Damage or irritation to the skin

surface promotes the release of these highly active molecules to act on adjacent epidermis and underlying connective tissue structures. Likewise, injury to the deeper dermal tissues can activate the expression of molecules that stimulate epidermal growth: the keratinocyte growth factors (FGF-7, -10). The extracellular matrix of intact and damaged tissue will also serve as a source for many bound growth factors as hydrolytic enzymes are released during the course of wound repair and remodeling. The breakdown of these structures is likely to release additional growth factor stores into the wound site. Indeed, extracellular matrix molecules may serve as an excellent delivery system for many different kinds of growth factors.

ANGIOGENESIS

The development of a new capillary bed, or angiogenesis, is a visible component of the proliferative phase of repair *(74)*. Neovascularization provides oxygen and nutrient support to the rapidly proliferating cells within healing wounds, and promotes granulation tissue formation. Angiogenesis in wounds follows an orderly and carefully regulated pattern. During the proliferative phase of wound healing, capillary growth continues until the capillary density reaches nearly three times that of uninjured normal tissue *(75)*. During the resolution phase of repair, most of the new capillaries regress, leaving behind a residual vascularity that is similar or slightly higher than uninjured tissue *(76)*. Such carefully regulated growth and regression of vessels occurs in adult mammals in only a few physiological circumstances, including wound healing, uterine cycling, follicular development in the ovary, and lactation in the female breast *(77)*. This pattern is in direct contrast to the dysregulated capillary growth that is a feature of many pathological diseases, including malignant tumors, retinopathies, and psoriasis *(78)*.

Neovascularization within wounds, as in all tissues, depends on many factors, including levels of growth factors, cell–cell interactions, cell–extracellular matrix interactions, and the activity of proteases *(79–81)*. One critical factor in all angiogenic processes is the balance between soluble proangiogenic and antiangiogenic factors that act directly on endothelial cells *(82)*. In both physiological angiogenesis, such as the healing wound, and pathological angiogenesis, such as malignant tumors, the net angiogenic stimulus at any particular point is believed to rely on the equilibrium between positive and negative mediators. Studies in healing wounds have demonstrated the importance of two key proangiogenic factors in stimulating this process. FGF-2 is abundant in wounds, as it is both released from its sequestered location within the extracellular matrix and actively synthesized in the healing wound *(83,84)*. FGF-2 levels diminish during the first few days after injury, at which time the dominant proangiogenic factor switches to vascular endothelial growth factor, or VEGF. VEGF is a potent, directly acting angiogenic factor that is capable of stimulating endothelial cell migration and activation in vitro, and angiogenesis in vivo *(85,86)*.

Vasculogenesis is a newer concept in wound healing. It is a process in which new blood vessels are formed by recruitment of precursors (endothelial progenitor cells) from the blood and hence the bone marrow. This phenomenon, together with the ability of VEGF to drive the process, offers an alternative method for creating new vasculature at sites of ischemia *(20,87)*. Angiogenesis has been described to be impaired in the healing wounds of diabetic animals, and some studies suggest that the topical application

of proangiogenic factors such as VEGF may improve healing outcomes in patients with diabetes *(88,89)*. Interestingly, though, several recent studies seem to indicate that robust angiogenesis is not a critical determinant of repair, and inhibition of angiogenesis alone does not appear to negatively impact healing *(90,91)*. One possible explanation for this puzzle might be that proangiogenic factors have effects well beyond a simple stimulation of endothelial cell growth and differentiation. Indeed, VEGF application to wounds has been shown to mobilize stem cells from the bone marrow *(89)*. In addition, several cell types other than endothelial cells, such as smooth muscle cells and leukocytes, have now been described to also have receptors for proangiogenic factors, and activation of these cells might influence healing *(92–95)*. In short, the application of proangiogenic factors may promote wound healing in many ways beyond enhanced vascularity.

FIBROPLASIA

The final phase of wound repair involves the formation of a scar. This occurs as the loosely woven, highly cellular granulation tissue gradually transforms into predominantly collagen-rich and less vascularized extracellular matrix. There is progressive reduction in capillary diameter and density, and the orientation of collagen fibers within granulation tissue will begin to change from a random organization to one that is more perpendicular to the wound site. Only after many weeks or months will collagen fiber organization eventually approach the concentration, orientation and fiber thickness of surrounding normal tissue. Thus it is quite easy to distinguish scar tissue at a microscopic level even after superficial signs of its presence have disappeared. Scar formation is often characterized by the phenomenon of wound contraction. This is a process that is driven by cellular elements within the connective tissue. Both fibroblasts and the more specialized myofibroblasts have contractile proteins within them that, under appropriate simulation, will act through integrin receptors to pull on the extracellular matrix and draw the margins of the wound toward one another. In pathological states, wound contracture occurs, which is a disfiguring and disabling wound repair phenomenon. A further characteristic of the scar formation phase of wound repair is the extensive remodeling of the extracellular matrix and cellular organization. The wound is hardly a static site. There is intense turnover of various components or building blocks of the system as the area transforms from a weak but efficiently formed fibrin clot into a strong but histologically distinguishable scar tissue. This remodeling is accomplished by the highly controlled expression of various extracellular matrix proteinases.

Traumatic injury can irreversibly destroy many structures in damaged tissue. In the skin, the extent of regeneration depends largely on the depth of the injury. Superficial injuries which only scrape off the upper layers of the epidermis will still leave behind many epidermal appendages such as sweat glands and hair follicles that can, in turn, serve as reservoirs for regeneration of new epidermis. As a consequence, superficial scrapes rapidly resurface not only from the wound margins but from all these internal sources. Deeper traumatic wounds or burns that remove or destroy these epidermal appendages result in the irreversible loss of these structures from the skin. Scar tissue is characterized by a more disorganized weave to the collagen fiber bundles, differences in capillary density, altered pigmentation of the epidermis, and a markedly diminished content and organization of elastic fibers.

PATHOBIOLOGY OF WOUND REPAIR

There are three highly significant pathologies that occur in the area of wound healing: nonhealing wounds, excessive healing, and the specialized response of tissues to burns. Nonhealing wounds or ulcers can arise from a variety of sources. Nevertheless, they share some important common features. First, ulcers all fail to develop an underlying connective tissue structure. Second, the lack of this important groundwork for cell organization results in impaired overgrowth of epithelium. Third, there is often reduced angiogenesis and reduced fibroplasia. Fourth, there is the persistent presence of inflammatory cells: neutrophils or macrophages. Fifth, because of this persistent failure to restore tissue integrity, the chronic wound is often a site of persistent bacterial infection, which in itself can stimulate inappropriate inflammatory cell responses.

There are a wide variety of clinical subtypes of nonhealing wounds which are not precisely enough distinguished at the biochemical level to allow classification. Many of these conditions arise as a result of transient or chronically impaired vascular supply. In the former case, local ischemia caused by pressure, other kinds of physical injury or even chemical injury can lead to the loss of oxygen and tissue perfusion, leading to local cell necrosis and tissue death. Reperfusion may exacerbate the injury. Under many circumstances, ischemia leads to the progressive replacement of damaged tissue by scar tissue with loss of important mechanical and physiological properties.

In the skin, nonhealing wounds classically form on the extremities and over bony processes as the result of either unrelieved pressure or repeated trauma. There is frequently a failure of both granulation tissue formation and epithelial overgrowth. Currently there is insufficient information on levels of active growth factors at sites of chronic nonhealing wounds. It is likely that either growth factor abundance or bioavailability is severely reduced in chronic wounds. Growth factor expression is reduced in diabetic wounds. For this reason a number of growth factors are being used in clinical trials to remedy nonhealing wounds.

The present evidence points to proteolytic degradation in the lesion environment as a major cause of wound-healing failure *(96)*. A variety of proteinases, including elastase and some of the metalloproteinases, are capable of degrading not only adhesive substrates for cell migration but also signaling molecules such as growth factors and cytokines *(97)*. In addition, excess proteolysis may cause a release of high levels of breakdown products of connective tissue that inappropriately activate inflammatory cell processes. Thus, much attention is being given to the development of appropriate proteinase inhibitors for control of certain forms of nonhealing wounds. It should be noted however, that necrotic tissue is an important negative factor in prolonging nonhealing wound. It is well recognized that extensive and aggressive debridement of such wounds is essential to stimulate healing. For this reason a number of manufacturers have developed protease formulations that have varying degrees of ability to discriminate between necrotic and living tissue.

Excessive Healing

Uncontrolled overgrowth at various phases of the repair process is an important aspect of wound repair pathobiology. Examples of wounds locked in the earliest phases of repair are pyogenic granuloma/pyoderma granulosum and other chronic inflammatory conditions of skin and other organs. Little is known about the mechanism which brings

about these conditions, but certainly many of the inflammatory states are owing to immunological stimuli.

Uncontrolled growth of scar tissue can take two forms. In hypertrophic scarring there is an excess growth of scar tissue within the wound margins, which may project well above the plane of the skin. These sites usually contain overabundant collagen and may be hypervascularized. Hypertrophic scars are commonly associated with second- and third-degree burns in which their lateral extent may provoke the formation of excessive contractures. Physical forces seem to play an important role in generating these types of scars, because coverage of injured areas with skin substitutes or splinting of the wound with a variety of films can often moderate the effects. Hypertrophic scars are known to contain fibroblasts that have abnormal growth factor responses and growth factor production profiles. It is quite likely that fibroblasts within the hypertrophic scar represent a subpopulation that has excess fibrotic tendencies owing to the overproduction of molecules such as TGF-β and a higher sensitivity to fibrogenic stimulation by this molecule. Presently, the only antagonist being evaluated in clinical trials is interferon-α. It is likely that further studies will attempt to address the problem more directly with TGF-β antagonists or perhaps molecules that block the action of the downstream effector, CTGF.

The other well-known cutaneous scar pathology is the keloid. This is an intriguing condition in which cutaneous injury results in scar formation that takes on the appearance of a benign tumor. Keloids grow beyond the lateral margins of the wound, and they are usually restricted in location to the upper trunk, face, neck, and ears. This regional variation emphasizes the heterogeneity of skin cell populations. There is also a predilection to keloid formation in many darker skinned races. There is also evidence of familial inheritance of the keloid tendency. Current studies are under way to attempt to map the keloid gene. The consequences of excessive healing in organs other than skin can be far more life threatening. Pulmonary, renal, and hepatic fibrosis all have marked effects of appropriate mechanical or secretory function in these tissues. The formation of surgical adhesions and is also an excess response to injury. Scarring occurs subsequent to ischemic injury of various organs and it leads to major health complications in coronary artery insufficiency and in stroke.

EXPERIMENTAL MODELS OF WOUND HEALING

Experimental Diabetes

The simplest method of creating a diabetic model in small animals is by chemical destruction of the pancreatic islets. In the rat and mouse, streptozotocin produces a rapid and profound hypoinsulinemia and hyperglycemia that is intended to mimic type 2 diabetes *(98)*. Animals cannot be maintained more than a few weeks to months in this state without insulin treatment, and wound healing is dramatically inhibited. Genetic models of type 2 diabetes include the db/db and ob/ob mouse mutations, which correspond to the deletion of the leptin receptor and leptin, respectively. Both of these mutants exhibit a high level of obesity that is consistent with the role of leptin in adipogenesis. Interestingly, leptin is expressed in wounds and it appears to have some anti-inflammatory properties *(56,99)*. Although both strains are diabetic, the lack of leptin signaling and the high obesity create a more complex physiological model. Other obesity models in the rat may hold more promise as type 2 diabetic analogs *(100)*. The nonobese

diabetic mouse *(101–104)* is considered a reasonable model of type 1 diabetes, because it is late in onset and linked to autoimmunity. However, it is a more expensive and time-consuming model because the disease occurs after several months. Although there are several other rodent strains that exhibit diabetic complications, they have not been routinely used for wound-healing evaluation.

Diabetes can also be induced in larger animals. Alloxan is the appropriate chemotherapeutic agent for pancreatic islet destruction in the rabbit. Interestingly, these animals are much more tolerant of the hyperglycemic state and survive several months without insulin therapy. The diabetic state has also been induced in pigs *(105)*.

Granulation Tissue and Angiogenesis

The filling and revascularization phases of wound repair are readily modeled in a variety of synthetic wounds. Among the most popular are so-called wound chambers, which can be either sponges, implantable cages made up of plastic or metal, or a sponge-like tissue equivalent into which cells will invade. This type of model has many advantages because it can define a wound space of fixed dimensions. In most of these models, trauma and hemorrhage is minimal, so that the wound repair process is initiated by exudation of plasma into the enclosed space followed by formation of a fibrin clot. Release of fibrin degradation products leads to subsequent, transient phases of inflammation granulation tissue formation and initiation of scar formation. Usually, such implants are less useful for studies of later phases of wound remodeling, except the capsule that surrounds such implants is a valuable index of tendency toward fibrosis.

Irritant Injection

The precursor to the wound chamber model was the implantation of materials, which induced a more robust inflammatory response. This includes a wide variety of foreign materials, including cotton pellets, carrageenan, and other irritants, or even simply the formation of an air bubble or blister. Because these are chronic inflammatory models, they are more strongly driven by neutrophil and macrophage-mediated processes than are the pure wound chamber models. They may be more useful therefore for studying pathobiologies associated with those inflammatory cells.

Implanted Biomaterials

A number of new biomatrix molecules have been used for specialized repair assays. These include collagen sponges which actually fit very nicely with the other wound chamber models, and a novel biomatrix known under the trade name of Matrigel™. This material is the product of secretion by embryonic chrondrosarcoma cells, and it contains most of the components of the basement membrane. It has been used by a number of laboratories as a substrate for identifying angiogenic events independent of fibroplasia. The drawback of Matrigel is that it not a highly purified material. Under normal circumstances, many cytokines and growth factor activities have been identified in the matrix of this biomaterial. Protocols that allow partial purification may reduce background problems.

Excisional Wounds

The excisional wound is, for many investigators still the *sine qua non* of wound-repair assays. A relatively large portion of epidermis and/or dermis is removed from one

or more sites on the experimental animal, and wound healing is monitored by various parameters, including the rate of closure of the wound, the rate of granulation tissue formation, and the changing biochemical character of granulation and scar tissue forming within the wound space. As discussed earlier, excisional wounds can be either full or partial thickness. Full-thickness wounds have more robust granulation tissue formation and heal only from the margins and the base. However, they also heal by contraction. Partial thickness wounds in animals will rapidly regenerate epidermis from remnant epidermal appendages, but the underlying, remnant dermis will act as a splint to prevent the artifact of wound contraction. Some investigators have taken advantage of wound contraction. In recent studies with recombinant mice, a full-thickness punch wound is made, and rates of contraction can be studied noninvasively over the course of several weeks. Because the mutant mice are usually a precious commodity, this is a useful assay. It is not clear how well differences in wound contraction predict to rates of healing in man.

For the study of epithelialization, one can also use the partial thickness injury as a good model. An alternative that is used often in transgenic and other mutant mice is stripping the tail skin with adhesive tape. This only damages the epidermis and leaves the dermis completely intact. The results may be analyzed by microscopic examination.

Incisional Biomechanics

The linear incision is still the key experimental tool for examining long-term effects of treatment on connective tissue formation. Incisions can be produced in a variety of sites and either left to close by secondary intention or closed with sutures or staples. Wound strength is then measured after excision of the injury site in a biomechanical testing device. Among useful parameters that can be derived are breaking strength, breaking energy, and elastic modulus. In addition, it is possible to gain some inference about the degree of cross-linking of the extracellular matrix by comparing the strength of wounds that have been preserved with or without formaldehyde fixation. The incision, at least that enclosed by primary intention, is not as suitable a model for examining histological effects of treatments, because the volume of granulation tissue is relatively small. Likewise, biochemical determinations are extremely difficult in this type of model.

Chronic Wounds

Generation of chronic wound models in a humane fashion is a challenge to the experimenter. For example, it has been extremely difficult to develop a model for true pressure sores in any laboratory species. Reports of success have been obtained by using greyhound dogs which have a very thin skin or using pigs in which the hindlimb is immobilized but placed in a cast that applies pressure. Constantine and Bolton have described an ischemic lesion that can be produced in guinea pigs by insertion of a rubber plug under the skin that is then ligated externally for several hours to produce a dermal infarct *(106)*. Chemical models may be somewhat easier to generate. Our laboratory, for example has capitalized on information first provided by Rudolph that the chemotherapeutic agent, adriamycin, produces a chronic lesion when injected into the dermis. Using this type of model, wounds in rats and rabbits persist for up to 60 days. A second model generated in this laboratory utilizes the toxicity of the venom of the brown recluse spider. This toxin causes the intense aggregation and eventual activation

of neutrophils at sites of injection, leading to a massive, localized hemorrhage and tissue necrosis. Such lesions also are extremely persistent, although the mechanism by which they develop is quite different from other burns. Thermal burns are certainly slow to heal in animal models. With high temperature burns, on has a combination of the effects of complete denaturation of the local site, coagulation of adjacent vascular supply by heat, and collateral, thermal effects that extend beyond the zone of acute damage. The extent of burning is easily controlled by temperature and the pressure of the applied heat source. Freezing burns or cryosurgical burns have a somewhat different nature, because the proteins of the killed tissue remain in a relatively native state. As a result there is quite a difference in terms of the kind of inflammatory reactions that occur in thermal as opposed to cryogenic burns. These burn models are most widely used to examine the effects of debriding agents because the major inhibitory effect is often the larger burden of excess tissue at the injury site.

SUMMARY

The process of wound healing is a complex cascade of cell interactions that leads to restoration of tissue integrity. In the diabetic extremity, healing is markedly slowed by a persistent inflammatory process and by reduced signaling and responsiveness of the target tissue. Healing is particularly compromised by poor macro- and microvascular circulation. Infection exacerbates the chronicity of the diabetic wound. Modern technologies are attempting to reverse this increasingly prevalent complication by surgical or pharmacological stimulation of vascularization, devices and molecules that promote tissue growth, and better understanding of the fundamental physiological features of the diabetic wound.

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